THE INHERITANCE OF CERTAIN MUTANT CHARACTERS IN VENTURIA INAEQUALIS [1]

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INTRODUCTION

Certain nonpathogenic Ascomycetes, such as *Neurospora sitophila* Shear and Dodge and *Neurospora crassa* Shear and Dodge, have been shown to offer exceptionally favorable material for genetic studies. The work of Dodge and his associates (*2*, *3*, *4*, *5*, *19*), [3] Lindegren and his coworkers (*10*, *11*, *12*, *13*, *14*, *15*), and others has greatly advanced knowledge of the mechanism of inheritance in this group of fungi. Keitt and his coworkers (*1*, *6*, *7*, *8*, *9*) have shown that the apple scab pathogen, *Venturia inaequalis* (Cke.) Wint., is very similar to the eight-spored species of *Neurospora* in adaptations for genetic work and affords favorable material for studies of inheritance of pathogenicity. These investigators have found only two types of pathogenic reaction, lesion and fleck, incited by monoascosporic lines freshly isolated from perithecia occurring in nature. They report that in all cases satisfactorily analyzed segregation of the factors for pathogenicity has occurred in 1:1 ratio, indicating that in a given isolate pathogenicity to a given variety is governed either by a single gene or by closely linked genes. They have also studied the cultural mutant characters *tan* and *nonconidial* and shown that the gene for either of these characters may suppress the expression of pathogenicity.

The present paper reports studies of inheritance of selected cultural mutant characters and sex reaction. Special attention was given to linkage relationships and to the effects of certain mutant genes on the expression of pathogenicity. A brief preliminary report of this work has been published (*17*). The background of the work and pertinent literature have been discussed in earlier papers (*6*, *8*).

MATERIALS AND METHODS

The mutants dealt with in this study arose in Petri dish cultures of monoascosporic lines of *Venturia inaequalis* studied by Keitt and Langford (*6*). All except *small* appeared as conspicuous sectors that grew more rapidly than the lines from which they arose. All have been described in more or less detail by Keitt and Langford (*6*) or Keitt, Langford, and Shay (*8*).

White (W).—This mutant arose as a sector (C 5 sector of Keitt and Langford) in isolate 5 of ascus C. It is pinkish white on malt agar (pl. 2, *A*) and produces conidia abundantly. In matings with *normal* it is highly fertile, producing abundant perithecia, the asci of

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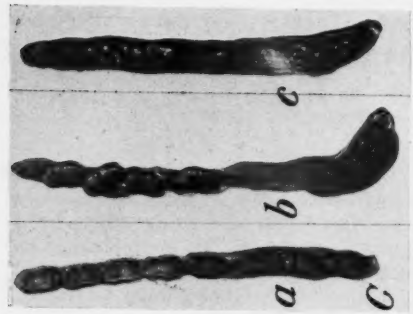
[3] Italic numbers in parentheses refer to Literature Cited, p. 40.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b
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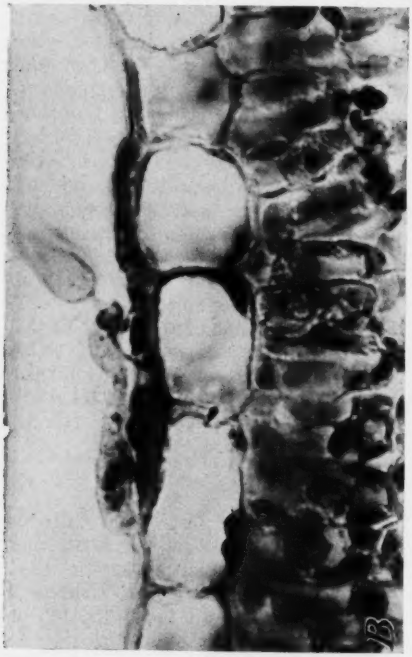
C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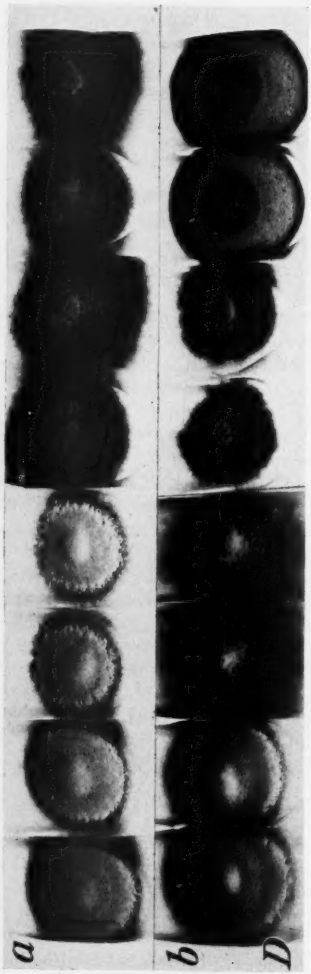
a
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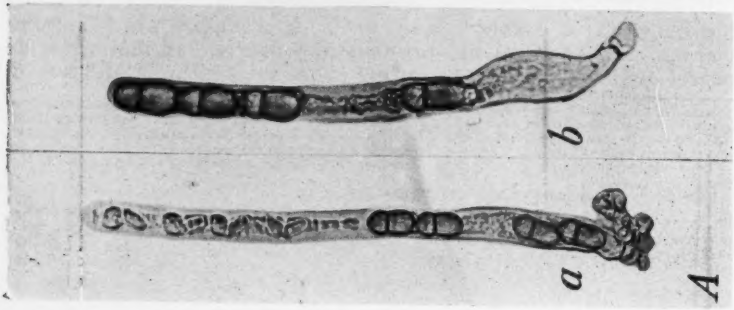
D


b
a
A

which contain four well-developed, *normal* ascospores and four that are partly or completely aborted (pl. 1, *A*). The partly aborted spores may be one- or two-celled and are usually misshapen and colorless. They are viable when sufficiently developed, and without exception the cultures grown from them have been *white*. In crosses of *white* × *normal*, white as well as typical dark ascocarps are produced, indicating that both the ascogonia and the antheridia produced in monoascosporic lines of this self-sterile hermaphroditic fungus are capable of functioning.

Tan (T).—This mutant arose as a sector (D 8 sector of Keitt and Langford) in isolate 8 of ascus D. It is characterized by tan color on malt agar and by producing conidia somewhat less abundantly than *normal* (pl. 1 *D, b*). Perithecia in crosses involving *tan* are produced sparsely, but the mutant has no aborting effect on the ascospores.

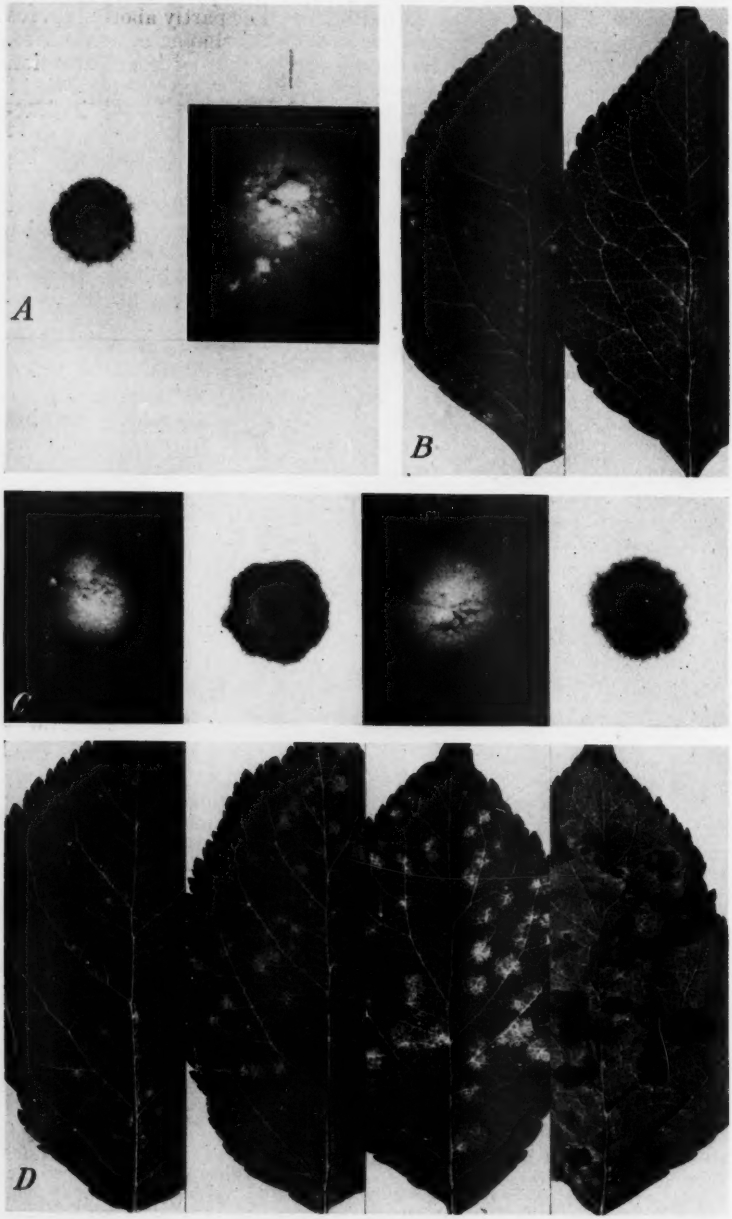
Nonconidial (Nc).—This mutant arose as a sector on the D 8 sector (*tan*) line of Keitt and Langford and was designated by them as sector from D 8 sector. It is characterized chiefly by the nearly complete suppression of conidial production and by its fine, compactly growing hyphae (pl. 1, *D, b*). On malt agar *nonconidial* cultures tend to be more grayish green than *normal* cultures.

Small (S). This mutant did not arise as a conspicuous sector but was discovered when an apparently *normal* sporulating isolate (one of eight *normal* lines taken 12 months previously from an ascus from an overwintered McIntosh leaf) was inoculated on McIntosh and failed to infect. Subsequent studies of the line established it as a mutant, but its use in this study was limited because it so nearly resembles *normal* in cultural characters, except for a somewhat smaller colony diameter, that it can be positively identified in the progeny only by the expensive and laborious process of testing pathogenicity.

In addition to the mutant characters, sex reaction (+ and −) was studied. The sex reactions of the lines are designated by the conventional +/− symbols. In previous work with *V. inaequalis* (*6*) sex reaction was tentatively designated by X and Y. The + sex reaction used throughout this paper is the X and the − is the Y

EXPLANATORY LEGEND FOR PLATE 1

A, Asci of *Venturia inaequalis* from a *white* × *normal* cross showing partial (*a*) and complete (*b*) abortion of ascopores carrying the *white* gene. *a*, × 429; *b*, × 531. *B*, The *small* mutant (line F 7) after 5½ days on McIntosh apple leaf, showing penetration mechanism and the restricted subcuticular development of the thallus. The outline of another conidium may be seen above the appressorium. × 600. *C*, *a*, eight-spored ascus typical of those from a *normal* × *normal* cross; *b*, four-spored ascus from a mating of a *normal* with a mutant, inducing complete ascospore abortion; *c*, ascus containing no spores, from a cross involving two compatible mutants each of which induces complete ascospore abortion. *a*, *b*, *c*, × 365. *D*, Sets of cultures from the ascospores of two asci from the *tan nonconidial* × *normal* mating, after 30 days in agar slants. The cultures are arranged in serial order with that arising from the No. 1 ascospore at the left. In *a*, it is shown that both *tan* and *nonconidial* segregated in the first meiotic division and were so distributed that four double mutants (*tan nonconidial*) and four *normal* cultures resulted. In *b*, it is seen that *tan* segregated in the first division and *nonconidial* in the second, giving the following distribution: 1, 2 *tan nonconidial*; 3, 4 *tan;* 5, 6 *normal;* 7, 8, *nonconidial*. Both *a* and *b* approximately natural size.


A
B
C
D

reaction reported by Keitt and Langford (*6*). Since none of the factors dealt with in this study are linked with sex reaction, there are no data from which conclusions as to the true nature of differentiation for sex reaction can be drawn. It is possible that future work will reveal that the +/-- factors herein treated as alleles are differentiated regions of a chromosome or that some other mechanism of differentiation for sex reaction exists. However, recent work in *Neurospora sitophila* (*18*), in which the gene *pink* is located distal to the sex factors, gives support to the allelomorphic +/− designation.

Normal denotes the typical, dark-colored, sporulating, wild-type culture.

The mutant and *normal* lines of *V. inaequalis* used in this study were carried in stock culture on Trommer's malt extract (2.5 percent) agar (1.7 percent). They were transferred at 8-week intervals by the monoconidial method (except in the case of *nonconidial* cultures, in which hyphal tips were used) described by Keitt and Langford (*6*).

The lines were mated in vitro and held at 6° to 8° C. after being at 20° C. for the first 10 days after seeding. Trommer's malt extract (0.5 percent) agar (2.5 percent) with a decoction of dead McIntosh apple leaves (25 gm. leaves per liter of medium) was used to obtain the perfect stage as described by Keitt and Langford (*6*). Usually about 4 months elapsed before the asci contained ripe ascospores suitable for isolation. When the asci were mature, the ascospores were isolated in serial order by the glass-needle method described by Keitt and Langford (*6*). After germination each ascospore was transferred to a test-tube slant of Trommer's malt extract (2.5 percent) agar (1.7 percent) and incubated at 16° C. for 4 weeks or until sufficient growth had occurred to permit determination of the cultural characters under investigation (pl. 1, *D*).

The method developed by Lindegren (*10*) for locating genes in Ascomycetes with reference to their centromeres or spindle-fiber attachments was used. One-half the percentage of asci showing second-division segregation for a given gene pair is taken as the uncorrected distance in cross-over units of the gene from its centromere. Lindegren's method has been criticized by Wülker (*20*) and Zickler (*21*). A discussion of these criticisms appears to be unwarranted in the present paper.

The pathogenicity tests were performed on trees in the greenhouse. The method of growing the trees and the inoculation technique used have been described by Keitt and Langford (*6*) and Keitt, Langford, and Shay (*8*).

EXPLANATORY LEGEND FOR PLATE 2

A, Parent isolates, *Venturia inaequalis*, D 2 *normal* (left) and C 5 sector *white* (right); monoconidial cultures, 8 weeks old. *B*, Red Astrachan leaves inoculated, respectively, with the isolates in corresponding positions in *A:* Left, flecking without sporulation; right, no macroscopic infection. *C*, Isolates representing the four biotypes (two *white* and two *normal*) from an ascus (XI) obtained from mating the lines shown in *A*. Isolates 1, 2, 5, and 6 (left to right), numbered in the order of occurrence in the ascus of the ascospores from which the cultures were derived; monoconidial cultures, 8 weeks old. *D*, Red Astrachan leaves inoculated, respectively, with the isolates in corresponding positions in *C;* two on left show flecking with no sporulation; two on right show sporulating lesions, one white and the other typically dark. *A* and *C*×ca. ½; *B* and *D*×ca. ¾.

EXPERIMENTAL RESULTS

LOCATION OF GENES

The percentages of second-division segregation for *white*, *tan*, *nonconidial*, *small*, and the sex reaction factors are shown in table 1. To determine whether the *white* gene pair segregated in the first or the second division, it was not necessary to dissect the asci but only to examine them microscopically. The arrangement of the nonaborted and the aborted spores could be readily and accurately determined (pl. 1, *A*). *White* and *tan* are nearest the centromere, being, respectively, 6.0 and 10.3 cross-over units distant. *Nonconidial* is relatively distant at 19.6 units from the centromere. The number of asci studied for the location of *small* is not sufficient to determine the location accurately, but the indications are that the gene is at a considerable distance from the centromere. The factors for sex reaction are also relatively far out on the chromosome arm. None of the distances given are corrected for double cross-overs.

Consideration was given to the possibility that passing of the two middle nuclei in the four-nucleate ascus (*15*, *18*) might occur in *Venturia inaequalis* and constitute a factor of error in these studies. Of the asci segregating for the *white* alleles, 88 percent showed segregation in the first division. If nuclear passing occurred frequently, there would be an excess of the alternate over the symmetric arrangement in those asci in which the arrangement of spores suggested second-division segregation. In a determination involving 169 asci showing second-division segregation of the *white* alleles, 80 showed the alternate (*w W w W* or *W w W w*) and 89 the symmetric (*w W W w* or *W w w W*) arrangement. Determinations in other crosses did not give evidence of any significant excess of the alternate arrangement in asci showing second-division segregation. Although the numbers of asci examined in these latter cases were not considered adequate, the data as a whole indicate that if nuclear passing occurs at the four-nucleate stage, it is not a factor of much importance in the studies reported in this paper.

TABLE 1.—*Summary of data on the distance from the centromere in cross-over units of several mutant genes and the sex reaction factors in Venturia inaequalis*

Character	Mating	Asci studied	Asci showing segregation in division—		Second division segregation	Distance of gene from centromere in cross-over units
			I	II		
		Number	*Number*	*Number*	*Percent*	*Number*
White	*W*×*w*	1,804	1,588	216	12.0	6.0
Tan	*T*×*t*	71	56	15	20.6	10.3
Tan	*T*×*t*	138	110	28		
Nonconidial	*Nc*×*nc*	138	84	54	39.1	19.6
Small	*S*×*s*	8	4	4	50.0	25.0
Sex reaction	+×−	43	18	25	58.1	29.1

LINKAGE RELATIONSHIPS

The associations of *white*, *tan*, *nonconidial*, and the sex-reaction factors as determined by the proportion of new to parental combinations appearing in the progeny of crosses involving these genes are

TABLE 2.—*Linkage relations of white, tan, nonconidial, and the sex-reaction factors in Venturia inaequalis*

Mating	Asci studied	Pairs of ascospores [1]	Progeny combinations		New combinations	Conclusions
			Parental	New		
	Number	*Number*	*Number*	*Number*	*Percent*	
W+×*w*−	41	164	70	94	57.3	Independent.
w+×*W*−	8	32	16	16	50.0	
T+×*t*−		260	120	140	53.8	Do.
Nc+×*nc*−		258	126	132	51.2	Do.
WT×*wT*	42	168	160	8	4.8	Linked.
TNc×*tnc*	138	552	288	264	47.8	Independent.

[1] The ascospores of each pair are genetically identical and constitute one of the 4 biotypes resulting from a meiosis.

shown in table 2. In most of the asci involving *white*, only four of the ascospores (*nonwhite*) were recovered, as all ascospores carrying *white* were partly or completely aborted and many were therefore nonviable. The genotype of the four ascospores bearing *white* could be accurately deduced from the four *nonwhite* ascospores recovered. For example, *tan* and *white* were found to be linked. Thus, an ascus from the *tan*×*white* cross in which the four nonaborted ascospores gave rise to two *normal* and two *tan* cultures would contain a cross-over. The four aborted ascospores in such an ascus, had they been recovered, would have given rise to two *white* and two *tan white* cultures. Similarly, the sex reaction of nonrecovered aborted ascospores could be deduced when that of the recovered nonaborted ascospores was ascertained.

Matings involving mutants are frequently much less fruitful than those involving only *normal* lines, and this unfruitfulness increases in cases of multiple mutants. For this reason the use of more than two or three mutant characters in a mating is difficult, for very few or possibly no perithecia are likely to result. Negative results were encountered on many occasions when *tan* and *nonconidial* lines were mated with *normal* of opposite sex reaction to determine whether these two mutant genes were linked with the sex-reaction factors. The data for complete asci were not always obtained, but the parental and new combinations that were obtained from a given ascus are recorded in table 2 by pairs of ascospores. For example, in an ascus containing *tan*, *tan nonconidial*, *nonconidial*, and *normal*, all but *normal* may fail to produce perithecia when tested for sex reaction. When the sex reaction of the *normal* pair of ascospores is determined, one of the *tan* pairs and one of the *nonconidial* pairs are known to be of the opposite sex reaction. Two pairs of ascospores from this ascus can then be recorded.

Because of the large numbers of trees that would have been required to identify *small* in the progeny, this character was not used in the linkage studies.

The data in table 2 show independent assortment for all genes studied except *tan* and *white*. The latter appear to be rather strongly linked with a distance of 4.8 cross-over units separating them. These data, in conjunction with the data from second-division segregation of these two gene pairs, permit the construction of a two-point chromosome map (fig. 1). *Tan* appears to be located at a distance of

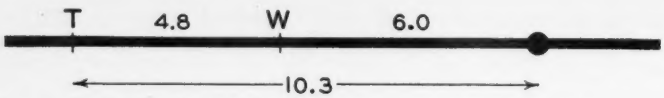


FIGURE 1.—Two-point map of the first chromosome in *Venturia inaequalis*.

10.8 units from the centromere and distal to *white* on what is here termed the first chromosome. The distance (10.3) of *tan* from the centromere as obtained from the frequency of second-division segregation is in good agreement with the sum (10.8) of the distance of *white* (6.0) as obtained from second-division segregation data (table 1) and the distance between *tan* and *white* (4.8) as determined by recombination (table 2). It can readily be seen that the data on recombination are from a source independent of these from second-division segregation.

PATHOGENICITY STUDIES OF *WHITE* AND *SMALL*

The original *white* mutant as reported by Keitt and Langford (*6*), and designated C 5 sector, was nonpathogenic to the Red Astrachan apple variety. This finding was confirmed in the present study. It is of interest, however, that the *white* progeny from a cross of *white*×*normal* are pathogenic. Results were obtained from but one ascus (designated ascus XI), of which the four biotypes are shown in plate 2. Three of the aborted *white* spores were viable, but the fourth (No. 8 in the ascus and paired with No. 5) failed to germinate. Considerable shifting of position occurred in the ascus, but this is not infrequent in asci containing aborted spores. The *normal* parent of the ascus (isolate 2 of ascus D of Keitt and Langford) incites the fleck reaction with Red Astrachan. The *normal* line from which *white* arose as a sector (isolate 5 of ascus C of Keitt and Langford) incites the sporulating lesion reaction with Red Astrachan. From the ascus of which the four biotypes are illustrated in plate 2, isolates 1, 2, 3, and 4 incite flecking on Red Astrachan; whereas 5, 6, and 7 incite sporulating lesions. Isolates 1, 4, and 5 are *white*. The No. 8 aborted (*white*) spore that failed to germinate would have incited sporulating lesions.

As stated earlier, the mutant *small* is nonpathogenic to all the varieties of apple on which it has been inoculated. Since the line produces abundant conidia in culture it became of interest to know at what point the mutant failed to establish pathogenicity. A histological study of the original mutant *small* on leaves of the McIntosh apple variety was undertaken, employing the methods used by Nusbaum and Keitt (*16*) for inoculating, killing, fixing, dehydrating, and embedding the tissue. Collections of inoculated leaf material were made at intervals up to 5½ days after inoculation. Heidenhain's iron-alum haematoxylin stain was used. Plate 1, *B*, shows the mutant *small* at 5½ days after inoculation. The fungus has penetrated in the typical way, but its subcuticular development has been sharply arrested, and is confined to a diameter of approximately three times the length of the conidium. This is in sharp contrast to the development of *normal* lines that incite the sporulating-lesion type of reaction (*16*). It is not known how much greater development of the incipient lesion would have occurred, but the readings of the results of inoculation studies using the progeny of *small*×*normal* were taken some 3 weeks subsequent to inoculation, and no macroscopic evidence of infection could be discerned.

DISCUSSION

The present work was undertaken as a step in exploring the possibility that genetic studies of cultural mutants of *Venturia inaequalis* may furnish information or materials of value in studies of inheritance of pathogenicity. The mechanism of inheritance in *V. inaequalis* appears to be essentially the same as that of the more extensively studied *N. crassa*. However, as has been pointed out in earlier papers, minor changes of position of nuclei or spores are not infrequently encountered in *V. inaequalis*. Usually the line of nuclear descent can be clearly deduced. In some cases, however, this is not possible. While these irregularities might preclude the use of this fungus for detailed studies of certain highly specialized genetic problems, such as chromatid interference in crossing over, they have not occasioned an important source of error for the studies reported in this paper.

The occurrence of the degenerate ascospore abortion character (pl. 1, *A*, *C*) is frequent among cultural mutants of *V. inaequalis*. Keitt and Langford (*7*) state: "Certain crosses of non-pathogenic sector lines with pathogenic lines [*normal*] gave 4-spore asci, whose 4 lines showed the same pathogenic reactions as the pathogenic parental line." Five of the nine cultural mutants of this organism so far studied (not all data have been published) induce ascospore abortion, a feature that has retarded progress of the genetic studies. Three of these five (including *white*) are pinkish white to white on malt agar; the other two are dark and not greatly different from a *normal* line in color on malt agar.

Of the genes studied, only *white* and *tan* were found to be linked. These are shown to be located on the same arm of the chromosome, 6.0 and 10.8 crossover units, respectively, from the centromere. Discovery of more linkage groups would be of interest in relation to the number of chromosomes of this species, which has not yet yielded to determination by cytological methods.

Different cultural mutants may have different effects on the expression of pathogenicity. The *tan* mutant, as shown by Keitt et al. (*8*), completely suppresses [4] any macroscopic pathogenic expression in every isolate-variety combination studied, Likewise, *small* suppresses macroscopic expression of pathogenicity, but as shown in plate 1, *B*, the fungus is able to penetrate and develop microscopically in the subcuticular position. As reported by Keitt et al. (*8*), *non-conidial* suppresses macroscopic expression of pathogenicity to all varieties studied except McIntosh, in which the sporulating-lesion reaction is reduced to a fleck. In the *white* mutant, as reported herein, there is suppression of macroscopic expression of pathogenicity of the C 5 *normal* line from which it arose as a sector; but, in the progeny stemming from a cross involving this mutant with *normal*, *white* does not affect the pathogenic reaction, though white instead of the typical dark conidia are produced on sporulating lesions. The basic reactions, sporulating lesion or fleck, are not at all affected. No explanation of this behavior of the *white* mutant based on experimental evidence can

[4] In this paper a gene is said to suppress the expression of pathogenicity when there is no evidence of pathogenicity in the lines in which the gene occurs, whereas there is normal expression of pathogenicity in the lines from which the gene has been segregated. The statement that a gene suppresses the expression of pathogenicity or determines any other character is not intended to imply that no other genes influence the expression of the character concerned.

now be advanced, but it is probable that modifying factors in the C 5 sector (*white*) line interacting with *white* suppressed the expression of pathogenicity. If so, it would follow that these modifiers segregated into the *normal* lines in ascus XI, since the *white* lines in that ascus are pathogenic.

These demonstrations that gene mutations are capable of complete or partial suppression of pathogenicity point to the importance of the elimination of modifying factors before a genetic analysis of pathogenicity is attempted.

SUMMARY

Four mutant genes and the sex-reaction factors in *Venturia inaequalis* (Cke.) Wint. have been located with reference to their centromeres. All characters studied were found to be independently inherited except *tan* and *white*. These two genes are located on the same chromosome arm, with *tan* 10.8 and *white* 6.0 cross-over units from the centromere.

The effects of a single mutation on the behavior of the fungus may be varied and profound. The *white* mutant prevents the development of the characteristic dark pigment in the thallus, induces abortion of ascospores, and probably by interaction with modifying factors suppresses the expression of pathogenicity in the line from which it arose. Another mutant, *small*, is shown to suppress macroscopic expression of pathogenicity, but penetration of the host is accomplished and a microscopic subcuticular development occurs.

The importance of giving adequate attention to the problem of modifying factors in parental material before undertaking an analysis of the inheritance of pathogenicity is emphasized.

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INHERITANCE OF REACTION TO SMUT, STEM RUST, AND CROWN RUST IN FOUR OAT CROSSES[1]

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INTRODUCTION

In the United States, the four major diseases of oats (*Avena* spp.) and their causal pathogens are loose smut (*Ustilago avenae* (Pers.) Jens.), covered smut (*U. levis* (Kell. and Sw. Magn.), stem rust (*Puccinia graminis avenae* Eriks. and Henn.), and crown rust (*P. coronata avenae* Eriks.). The most satisfactory and in some instances the only way of controlling these diseases is by using resistant varieties. New disease-resistant varieties of oats possessing high yielding ability and other desirable agronomic characters are being produced rapidly through plant breeding. Knowledge concerning the mode of inheritance of disease reaction greatly facilitates such work. The investigations reported here were undertaken to determine the mode of inheritance of reaction to the above diseases in certain crosses involving red oats.

MATERIAL AND METHODS

The four crosses used in this study were made in the greenhouse at Manhattan, Kans., during the winter of 1939–40. Richland-Fulghum (Kans. 6155) was used as one of the parents in each of the crosses. The other parents were Anthony-Bond (Iowa 1826), Fulghum-Victoria (C. I.[3] 3485), and Fultex (C. I. 3531). Fultex is a selection from a cross between Fulghum and Victoria. These parents derived from hybrids were advanced lines that were presumably homozygous for agronomic characters and reaction to disease. Their disease reactions to the physiologic races of the rust and smut organisms used in this study are shown in table 1. The crosses studied were Richland-Fulghum × Fulghum-Victoria and reciprocal, Fultex × Richland-Fulghum, and Anthony-Bond × Richland-Fulghum.

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[2] Appreciation is expressed to L. P. Reitz and L. E. Melchers for their assistance and suggestions throughout the investigation, and to Dr. E. C. Stakman, Minnesota Agricultural Experiment Station, for supplying a culture of race 8 of stem rust for the greenhouse studies.

[3] C. I. refers to accession number of the Division of Cereal Crops and Diseases.

TABLE 1.—*Reaction of parent lines to the physiologic races of the smut, stem rust, and crown rust organisms used as inoculum in studies on the inheritance of reaction to disease, Manhattan, Kans., 1941–42*

Parent line or variety	Identification No.	Inoculum							
		Ustilago avenae and U. levis		*Puccinia graminis avenae*				*P. coronata avenae*	
		Composite of races		p. r.[1] 2		p. r. 8		p. r. 1	
		Percent infection	Response	Infection type	Response	Infection type	Response	Infection type	Response
Richland-Fulghum	Kans. 6155	0	R[2]	2	R	4	S	4	S
Fulghum-Victoria	C. I. 3485	0	R	4	S	(3)	.....	2	R
Fultex	C. I. 3531	0	R	4	S	(3)	.....	2	R
Anthony-Bond	Iowa 1826	5.0	MR	2	R	2	R	0	R

[1] p. r. = physiologic race.
[2] R = resistant, MR = moderately resistant, S = susceptible.
[3] Not tested for reaction to physiologic race 8.

The F_1 plants were grown in the greenhouse at Manhattan, Kans., during the winter of 1940–41. Part of the seed from each F_1 plant was sent to the Aberdeen Substation, Aberdeen, Idaho, where part of the F_2 generation was grown under irrigation for seed increase purposes. The remainder was inoculated by dusting the seed with a composite of chlamydospores of several races of *Ustilago avenae* and *U. levis* known to attack the varieties Kanota and Richland and of a race of *U. avenae* known to attack the variety Fulton in Kansas. These races probably were representative of the smut organisms existing in Kansas. This seed was then space-planted in the disease nursery at Manhattan. It is recognized that, from a purely genetic point of view, the use of composite inoculum containing several physiologic races of two species of *Ustilago* is open to criticism. However, the present study was designed for its plant breeding as well as its genetic aspects. From the standpoint of practical plant breeding, resistance to all races and species was the objective. Fortunately the results obtained can be explained on a relatively simple genetic basis.

Artificial epiphytotics of crown rust and stem rust were produced in the Manhattan disease nursery by the hypodermic-syringe method of inoculating susceptible plants in uniformly distributed check and rust-spreader rows. Spore suspensions were injected into the curled leaves of developing plants. Crown rust inoculations were begun 2 weeks in advance of the stem rust inoculations. Race 1 of *Puccinia coronata avenae* and race 2 of *P. graminis avenae* were used in all of the field investigations since they were the most common races of these organisms occurring in Kansas. Each F_2 plant grown at Manhattan was examined for crown rust, stem rust, and smut at the proper time and the reaction recorded. The reactions of the resistant and susceptible parents were used as a basis for classification.

The F_3 generations studied consisted of the progeny of the F_2 plants grown at Manhattan and also at Aberdeen. The rust reactions of the F_3 generations were studied in the seedling stage in the greenhouse

during the winter and in the adult plant stage in the field the following spring. In the greenhouse each F_3 line was planted in a 2½-inch flowerpot at the rate of approximately 30 seeds per pot. Separate plantings were made for crown rust and stem rust studies. The recorded seedling reaction was that of the primary leaf only. Seedlings were inoculated 7 days after planting by placing the plants in a moist chamber, spraying them with water, and then dusting with spores. A 24-hour period in the moist chamber at ordinary greenhouse temperatures of 70° to 75° F. was found to be sufficient for infection by either rust organism. Approximately 7,000 pots of seedlings were inoculated and observed during the winter. Three pots of each parent were planted and inoculated with each lot of hybrids, giving a constant check on the intensity of inoculation and the race purity of the rust cultures. Race 1 of *Puccinia coronata avenae* and races 2 and 8 of *P. graminis avenae* were used in the greenhouse investigations. Extreme care was used in keeping all of the rust cultures pure. Differential varieties were inoculated with each race at 2-week intervals during the studies to check race purity.

Approximately 30 seeds of each F_3 line were inoculated with the smut composite and planted in the disease nursery in 1942. Smut inoculation consisted of adding spores to the seed envelopes and shaking vigorously. The seed was not dehulled. Stem rust and crown rust epiphytotics were produced in the same manner and with the same races of the rust organisms as described for the F_2 field studies in 1941. Satisfactory readings were obtained on crown rust and smut reaction but stem rust infection was neither sufficiently heavy nor distributed uniformly enough for satisfactory records in 1942.

EXPERIMENTAL RESULTS

INHERITANCE OF REACTION TO SMUTS

Studies on the inheritance of reaction of oats to the smut organisms have been made by several investigators and the reported results have indicated one, two, or three factors governing resistance, depending on the varieties used as parents. Some of the recent studies involving the varieties Bond and Victoria are particularly pertinent to the present studies. Hayes, Moore, and Stakman [4] and Hayes [5] reported that the smut reactions of the crosses Bond × Anthony and Bond × Iogold were determined by one factor with resistance dominant. The action of two dominant factor pairs, one a factor for high resistance, the other a factor for partial resistance, was observed by Torrie [6] in the crosses Iowa 444 × Bond, Victoria × Richland, and (Victoria-Richland) × State Pride.

The results reported here on the inheritance of resistance to smut agree with published results in some respects but differ from them in others. Although all of the parental lines except Anthony × Bond were known to be highly resistant to smut in Kansas, seed for

[4] HAYES, H. K., MOORE, M. B., and STAKMAN, E. C. STUDIES OF INHERITANCE IN CROSSES BETWEEN BOND, AVENA BYZANTINA, AND VARIETIES OF A. SATIVA. Minn. Agr. Expt. Sta. Tech. Bul. 137, 38 pp., illus. 1939.

[5] HAYES, H. K. BREEDING FOR RESISTANCE TO CROWN RUST, STEM RUST, SMUT, AND DESIRABLE AGRONOMIC CHARACTERS IN CROSSES BETWEEN BOND, AVENA BYZANTINA, AND CULTIVATED VARIETIES OF AVENA SATIVA. Amer. Soc. Agron. Jour. 33 : 164–173, illus. 1941.

[6] TORRIE, J. H. CORRELATED INHERITANCE IN OATS OF REACTION TO SMUTS, CROWN RUST, STEM RUST, AND OTHER CHARACTERS. Jour. Agr. Res. 59 : 783–804, illus. 1939.

the F_2 grown at Manhattan and for the F_3 from F_2 plants grown at Aberdeen and Manhattan was inoculated and sown in the nursery. The seed for F_2 was dehulled before smutting but seed for F_3 was smutted without dehulling. Check rows of the parents were grown at regular intervals in the nursery each year. The seed was inoculated and grown in the same manner as the hybrid lines. At heading time definite segregation was noted in both F_2 and F_3 despite the fact that both parents of all but one cross were highly resistant. F_2 plants wholly or partially smutted were classified as smutted, whereas in F_3 a line was considered smutted if one or more smutted plants appeared in the row. Only F_3 lines yielding no smutted plants were classified as resistant. In F_2 all but four of the smutted plants were only partially smutted so it was possible to grow F_3 lines from them.

Higher infection was obtained in F_3 lines from F_2 plants grown at Manhattan than in lines from similar plants grown at Aberdeen. The first three crosses had an average of 45.8 percent of the F_3 lines infected when grown from Manhattan seed and 32.4 percent from Aberdeen seed. The F_3 lines of the fourth cross had 43.3 percent and 17.5 percent infection, respectively. The reason for this is not definitely known. It was noted that seed grown under irrigation at Aberdeen was much larger and plumper than that grown under dry conditions at Manhattan. It also was noted both in the greenhouse and in the field that seedlings grown from Aberdeen seed were much more vigorous than those grown from Manhattan seed. It is possible that the poorer vigor of seedlings from Manhattan seed was associated with higher smut infection. A more important factor probably was the difference in smut conditions under which the F_2 plants were grown. The seed planted at Aberdeen was not smutted and the F_2 plants at that station were smutfree. At Manhattan the seed for F_2 was smutted and there were some smutted plants in the population. In addition, there was heavy smut in susceptible check rows of Kanota oats grown with the F_2 at Manhattan. This may have resulted in much natural infection in the field. Such possibilities suggest that the Manhattan F_3 lines probably give a more complete record of the actual inheritance of smut reaction than do lines from Aberdeen seed since there was less escape from infection at Manhattan. The data obtained from the Manhattan lines, therefore, were used in calculating the manner of inheritance, and the data on this factor are recorded in table 2.

TABLE 2.—*Segregation for reaction to smut noted in F_2 and F_3 generations of 4 oat crosses grown in the field at Manhattan, Kans.*

Cross	F_2 reaction		F_3 reaction	
	Plants observed	Plants smutted	Lines observed	Lines smutted
	Number	*Percent*	*Number*	*Percent*
Richland-Fulghum×Fulghum-Victoria	104	9.6	104	55.8
Fulghum-Victoria×Richland-Fulghum	338	3.6	337	41.8
Fultex×Richland-Fulghum	133	10.5	133	48.1
Summary of 3 R–F×F–V crosses	575	6.26	574	45.8
Anthony-Bond×Richland-Fulghum	940	1.1	938	43.3

The parental lines Fulghum-Victoria, Fultex, and Richland-Fulghum were highly resistant to the smut composite, showing no

infection. The F_2 and F_3 generations of the crosses involving these resistant parental lines showed transgressive segregation for smut susceptibility. The F_2 generations of the three crosses involving Richland-Fulghum and Fultex or Fulghum-Victoria averaged 6.26 percent smutted plants. In the F_3, 45.8 percent of the lines of these crosses grown from Manhattan seed contained smutted plants.

Apparently each parent carries a separate dominant factor pair for resistance to smut. The presence of either or both of these factors in heterozygous or homozygous condition results in resistance to smut. The factors, although different, produce the same result and therefore are considered duplicate factors. A ratio of 15 resistant to 1 susceptible plant would be expected in F_2, only those hybrid plants lacking both dominant factors being susceptible. If this hypothesis is correct, 6.25 percent of the F_2 population would be expected to be smutted; actually 6.26 percent were observed. In the F_3, 56.25 percent of the lines would be expected to show smutted plants. On this basis 7 F_3 lines would breed true for resistance, 8 would segregate (and therefore would contain smut), and 1 would breed true for susceptibility to smut. Actually 55.8 percent of the lines in 1 of the crosses were smutted. Lower percentages of infection in the other crosses probably can be accounted for by escape from smut infection as there were only 15 to 30 plants representing each line and the seed was not dehulled. The data and discussion presented concerning the crosses involving Fulghum-Victoria, Fultex, and Richland-Fulghum indicate that smut resistance in these crosses is dominant and due to 2 main factor pairs.

The Anthony-Bond parent was moderately resistant to smut, showing less than 5 percent infection, while the Richland-Fulghum parent showed no infection to the smut inoculum used. The F_2 generation had 1.1 percent of the plants smutted while 43.3 percent of the F_3 lines were smutted. These results are similar to those obtained in the other three crosses. However, in the Anthony-Bond × Richland-Fulghum cross it is assumed that there are two dominant factor pairs for resistance, one of which is a factor for high resistance, the other a factor for moderate resistance.

INHERITANCE OF REACTION TO STEM RUST

Resistance to stem rust has been found by many investigators to be dominant and inherited on a single factor basis. Recent reports on the inheritance of resistance in cases involving the Bond and Victoria varieties are particularly pertinent to the studies reported herein. Hayes, Moore, and Stakman [7] and Hayes [8] found that reaction to *Puccinia graminis avenae* (physiologic race 1) was governed by one factor with resistance dominant in seven crosses when Bond was the susceptible parent. Torrie [9] observed similar inheritance in eight crosses involving susceptible Bond and Victoria when inoculated with races 2, 5, and 7 of *P. graminis avenae*.

The mature plant reactions of the F_2 generation in the field and the seedling reactions of the F_3 generation in the greenhouse to race 2 of *Puccinia graminis avenae* were studied at Manhattan for the crosses Richland-Fulghum × Fulghum-Victoria, Fulghum-Victoria

[7] See footnote 4, p. 45.
[8] See footnote 5, p. 45.
[9] See footnote 6, p. 45.

× Richland-Fulghum, and Fultex × Richland-Fulghum. A summary of the segregation for stem rust reaction in the F_2 and F_3 generations is given in table 3. The F_2 data are based entirely on plants grown at Manhattan; the F_3 data are based on the progeny of F_2 plants grown both at Manhattan and at Aberdeen.

The Fulghum-Victoria and Fultex parents were susceptible to race 2. Uredia on these susceptible parents were large, linear, and erumpent, producing many spores (fig. 1). On the other hand, the resistant Richland-Fulghum parent had small, circular, inconspicuous uredia which produced relatively few spores. The F_1 plants were almost as resistant as the Richland-Fulghum parent. Two types of rust reaction were observed in the F_2 plants. One phenotype resembled the resistant Richland-Fulghum parent while the other was as susceptible as the Fulghum-Victoria and Fultex parents. Of 575 plants of the 3 crosses grown in the field at Manhattan, 407 were resistant and 168 were susceptible. Resistance therefore appears to be dominant in the F_2 and to be due to a single main-factor difference. The F_3 progeny of these plants were tested in the seedling stage in the greenhouse. With but 8 exceptions the progeny from susceptible F_2 plants bred true for susceptibility in the F_3 generation. Seedlings of 33 segregating F_3 lines of Fulghum-Victoria × Richland-Fulghum and 14 segregating lines of Fultex × Richland-Fulghum were classified for rust reaction. The counts (table 3) revealed segregation closely approximating the 3 to 1 ratio observed in F_2.

The data presented indicate that in these crosses the resistance of Richland-Fulghum to physiologic race 2 is dominant and due to a single main-factor pair.

The fourth cross in which resistance to stem rust was studied was Anthony-Bond × Richland-Fulghum. The primary purpose in making this cross was to obtain segregates having combined resistance to physiologic races 2 and 8 of *Puccinia graminis avenae* and race 1 of *P. coronata avenae*. Both parents of the cross proved to be resistant to race 2 while Anthony-Bond was resistant and Richland-Fulghum was susceptible to race 8. Physiologic race 2 was used to inoculate F_2 plants in the field at Manhattan. No segregation was obtained, all of the plants being as resistant as the two parents. Inoculations with physiologic race 8 were made only on seedling plants of the F_3 generation in the greenhouse because that race has not been common in natural infections in the vicinity of Manhattan. The reaction of Anthony-Bond to physiologic race 8 was characterized by very small circular pustules surrounded by "green islands" of chlorophyll. Richland-Fulghum was suspectible to this race as shown by the presence of large, linear pustules. Figure 2 shows the seedling reaction of the parents and of hybrid lines of this cross to physiologic race 8 of *P. graminis avenae*.

By growing the progeny of F_2 plants produced both at Manhattan and Aberdeen, 1,272 F_3 lines were available for study. Of this number, 324 lines were observed to be breeding true for resistance, 632 lines were segregating, and 316 lines were breeding true for susceptibility. These were close to the expected numbers on the basis of a single factor difference (table 3). Counts also were made in 52 of the segregating lines and revealed a ratio of 1,811 resistant to 579 susceptible plants.

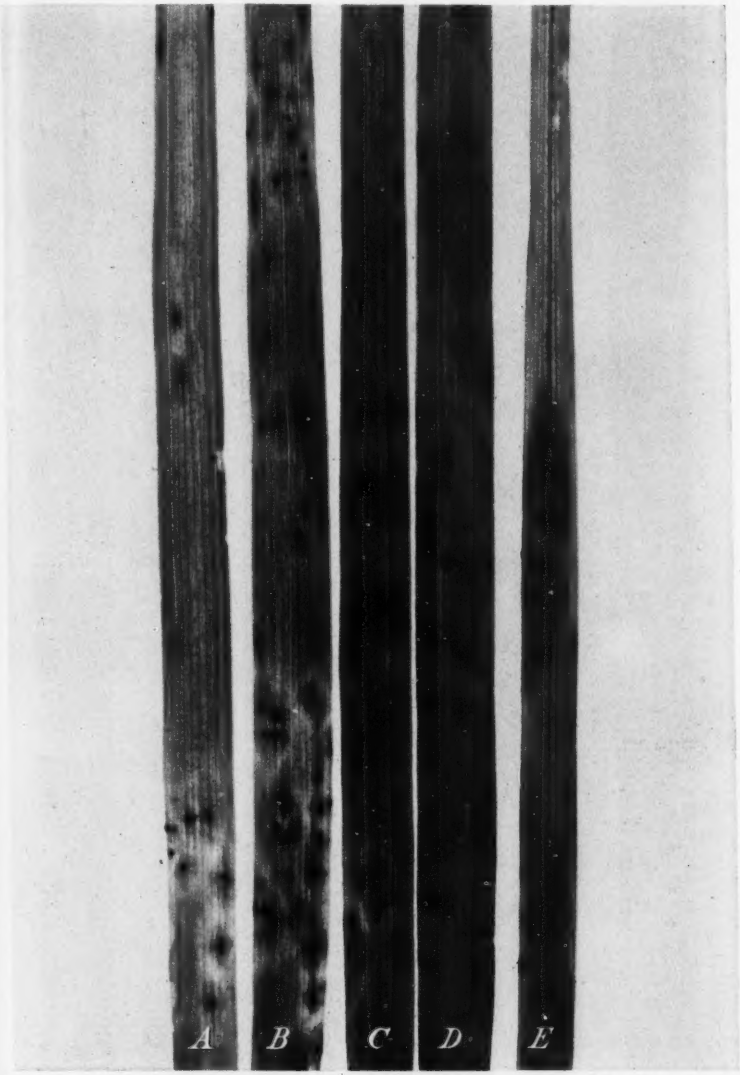

FIGURE 1.—Reaction of primary leaves of seedling plants to *Puccinia graminis avenae* physiologic race 2 in the cross Richland-Fulghum × Fulghum-Victoria and in the two parental lines: *A*, resistant Richland-Fulghum parent; *B* and *C*, resistant F_3 lines; *D*, a susceptible F_3 line; *E*, the susceptible Fulghum-Victoria parent.

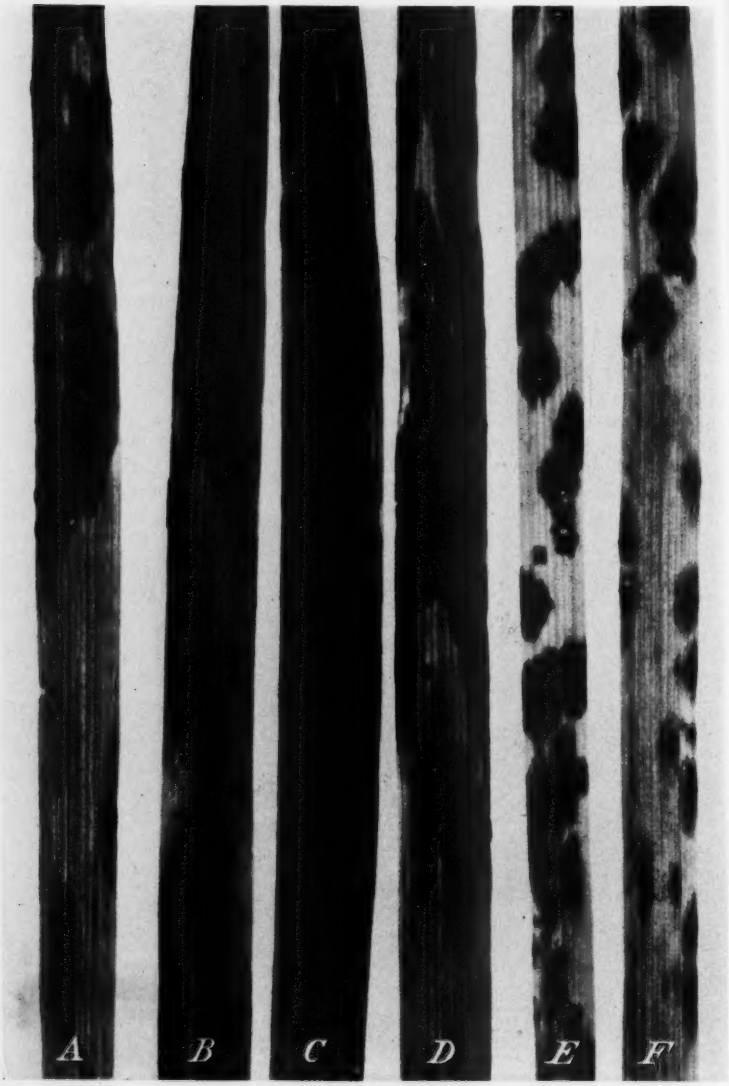

FIGURE 2.—Reaction of primary leaves of seedling plants of the cross Anthony-Bond × Richland-Fulghum and of parental lines to *Puccinia graminis avenae* physiologic race 8; *A*, susceptible Richland-Fulghum parent; *B*, a susceptible F_3 line; *C* and *D*, types of intermediate F_3 lines; *E*, a resistant F_3 line; *F*, the resistant Anthony-Bond parent.

TABLE 3.—*Segregation for reaction to Puccinia graminis avenae in 4 oat crosses in mature F_2 plants in the field and seedlings of F_3 lines grown in the greenhouse; the first 3 crossee were inoculated with physiologic race 2 and the last with race 8*

Cross	Observed or expected	F_2 generation in field					F_3 generation in greenhouse						Segregation in segregating F_3 lines					
		Number of plants			χ^2	Range of P	Number of lines				χ^2	Range of P	Number of lines counted	Number of plants			χ^2	Range of P
		R	S	Total			R	Seg.	S	Total				R	S	Total		
Richland-Fulghum × Fulghum-Victoria	O	73	31	104	1.282	0.20–0.30	22	65	33	120	2.850	0.20–0.30						
	E	78	26				30	60	30									
Fulghum-Victoria × Richland-Fulghum	O	237	101	338	4.024	.01–.05	151	346	227	724	17.370	.01	33	1,104	385	1,489	0.606	0.30–0.50
	E	253	85				181	362	181					1,117	372			
Fultex × Richland-Fulghum	O	97	36	133	.363	.50–.95	66	186	101	353	7.878	.01–.05	14	482	163	645	.033	.50–.95
	E	100	33				88	177	88					484	161			
Summary of R–FX F–V crosses	O	407	168	575	5.336	.01–.05	239	597	361	1,197	24.903	.01	47	1,586	548	2,134	.490	.30–.50
	E	431	144				299	599	299					1,600	534			
Anthony-Bond × Richland-Fulghum	O	940	0	940			324	632	316	1,272	.151	.50–.95	52	1,811	579	2,390	.723	.30–.50
	E						318	636	318					1,793	597			

It appears, therefore, that resistance to race 8 of stem rust is dominant and is controlled in this cross by one factor which differs from that governing resistance to race 2 in the other crosses.

INHERITANCE OF REACTION TO CROWN RUST

Murphy, Stanton, and Stevens,[10] and Weetman [11] reported that the inheritance of the crown rust resistance of Victoria is controlled by one dominant genetic factor. The inheritance of the crown rust resistance of Bond was found to be governed by two dominant complementary factors by Hayes, Moore, and Stakman,[12] Hayes,[13] and Weetman.[11] Torrie [14] suggested that the crown rust reaction in the cross Iowa No. 444 × Bond was governed by two factor pairs, one a factor for crown rust resistance and the other a partial inhibitor of the factor for resistance.

The reaction of the F_2 generation of all four crosses to race 1 of *Puccinia coronata avenae* was studied at flowering time in the field in 1941. The reaction of the F_3 generation of the same crosses was studied in the seedling stage in the greenhouse during the following winter and in the mature plant stage in the field in 1942. Pure cultures of physiologic race 1 were used in all inoculations.

A strain of Richland-Fulghum was used as the susceptible parent in each of the four crosses. This strain has large normal uredia unaccompanied by chloronemia. The Fulghum-Victoria and Fultex parents were resistant, having small, nearly circular pustules surrounded by the necrotic areas so characteristic of the Victoria type of resistance. Severe seedling infection of this type caused a complete drying of the infected leaves of resistant plants, giving the seedlings a blighted appearance. Typical seedling reactions of the parents and of the F_3 hybrids of the crosses involving the Victoria type of resistance are shown in figure 3. Anthony-Bond, the resistant parent of the fourth cross, exhibited the near-immunity of Bond.

Since the two types of resistance to crown rust race 1 proved to be inherited in different ways, they will be discussed separately. The manner of inheritance of the Victoria type of resistance will be considered first. The F_1 plants were nearly as resistant as the Fulghum-Victoria and Fultex parents. Three different types of rust reaction were observed in mature plants of the F_2 generation in the field.

One phenotype resembled the resistant parent, another resembled the susceptible parent, and a third type was intermediate, duplicating the F_1 reaction. Owing to their near-resistance the intermediate types and resistant types were grouped together and compared with the susceptible. Reaction of the F_2 and F_3 generations determined on that basis are shown in table 4. In the F_2 generation, segregation appeared to be 3 resistant plants to 1 susceptible, while the F_3 segregated 1 resistant line, to 2 segregating lines, to 1 susceptible line. Thus

[10] MURPHY, H. C., STANTON, T. R., and STEVENS, H. BREEDING WINTER OATS RESISTANT TO CROWN RUST, SMUT, AND COLD. Amer. Soc. Agron. Jour. 29 : 622–637. 1937.
[11] WEETMAN, L. M. GENETIC STUDIES IN OATS OF RESISTANCE TO TWO PHYSIOLOGIC RACES OF CROWN RUST. (Abstract) Phytopathology 32 : 19. 1942.
[12] See footnote 4, p. 45.
[13] See footnote 5, p. 45.
[14] See footnote 6, p. 45.

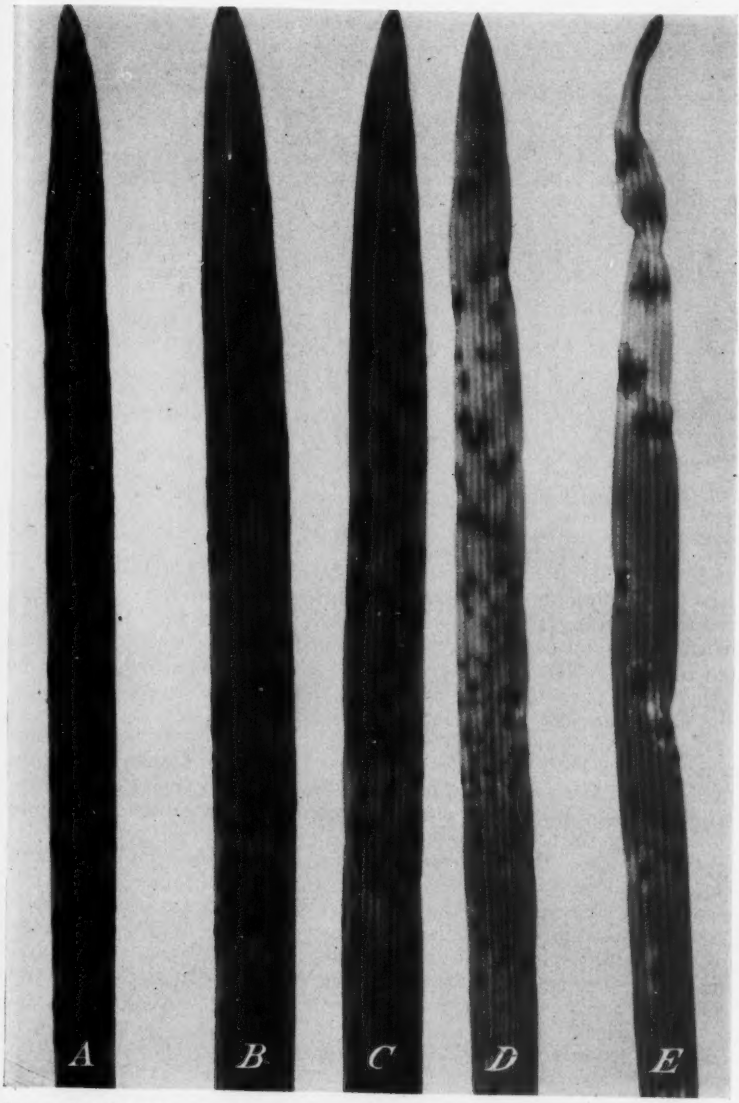

FIGURE 3.—Reaction of primary leaves of seedling plants of the cross Richland-Fulghum × Fulghum-Victoria and of parental lines to physiologic race 1 of *Puccinia coronata avenae: A*, the susceptible Richland-Fulghum parent; *B*, a susceptible F_3 line; *C*, an intermediate F_3 line; *D*, a resistant F_3 line; *E*, the resistant Fulghum-Victoria parent.

resistance to crown rust in the three crosses involving Fulghum-Victoria and Fultex was partially dominant and controlled by one factor. There was good agreement between field and greenhouse F_3 observations and there appeared to be no difference in the crown rust reaction of seedlings and mature plants in the progeny of these crosses.

TABLE 4.—*Segregation in 3 oat crosses for reaction to Puccinia coronata avenae physiologic race 1 observed in the mature F_2 plants in the field and seedlings of F_3 lines grown in the greenhouse*

Cross	Observed or expected	F_2 generation in field					F_3 generation in greenhouse					
		Number of plants			χ^2	Range of P	Number of lines				χ^2	Range of P
		R	S	Total			R	Seg	S	Total		
Richland-Fulghum × Fulghum-Victoria	O	65	39	104	8.667	0.01	31	45	44	120	10.317	0.01
	E	78	26				30	60	30			
Fulghum-Victoria × Richland-Fulghum	O	258	80	338	.393	.50–.95	178	368	178	724	.199	.50–.95
	E	253	85				181	362	181			
Fultex × Richland-Fulghum	O	100	33	133	.000	1.00	76	173	104	353	4.636	.05–.10
	E	100	33				88	177	88			
Summary of R-F × F-V crosses	O	423	152	575	.593	.30–.50	285	586	326	1,197	3.376	.10–.20
	E	431	144				299	599	299			

The inheritance of resistance to crown rust in Anthony-Bond × Richland-Fulghum was different from that encountered in the other crosses. Anthony-Bond carries the high resistance of Bond to most of the prevalent races of *Puccinia coronata avenae* including physiologic race 1. The only symptom of crown rust infection observed in hybrid and parental plants carrying that type of resistance was a slight flecking where infection occurred (fig. 4).

Plants in F_1 exhibited at the four- to five-leaf stage an intermediate reaction. In F_2 it became evident that the inheritance of resistance in this cross was more complex than that found in the other crosses. F_2 plants were grown in the field and crown rust readings were made at flowering time. The plants were divided into resistant and susceptible groups. The resistant group was subdivided into two more or less distinct classes, one of which was highly resistant, showing only slight flecking, while the other was moderately resistant, exhibiting slight pustule development and heavy flecking. The susceptible group also was subdivided into two groups, one fully susceptible and the other intermediate but approaching full susceptibility. In many cases it was difficult to determine whether a plant should be classed as fully susceptible or as intermediate. A similar difficulty sometimes was experienced in distinguishing between highly resistant and moderately resistant plants. There were 310 plants of the F_2 generation classified as resistant. Of this group 120 plants were classed as highly resistant and 190 as moderately resistant. In the susceptible group there were 630 plants, 158 of which were classed as intermediate and 472 as fully susceptible (table 5).

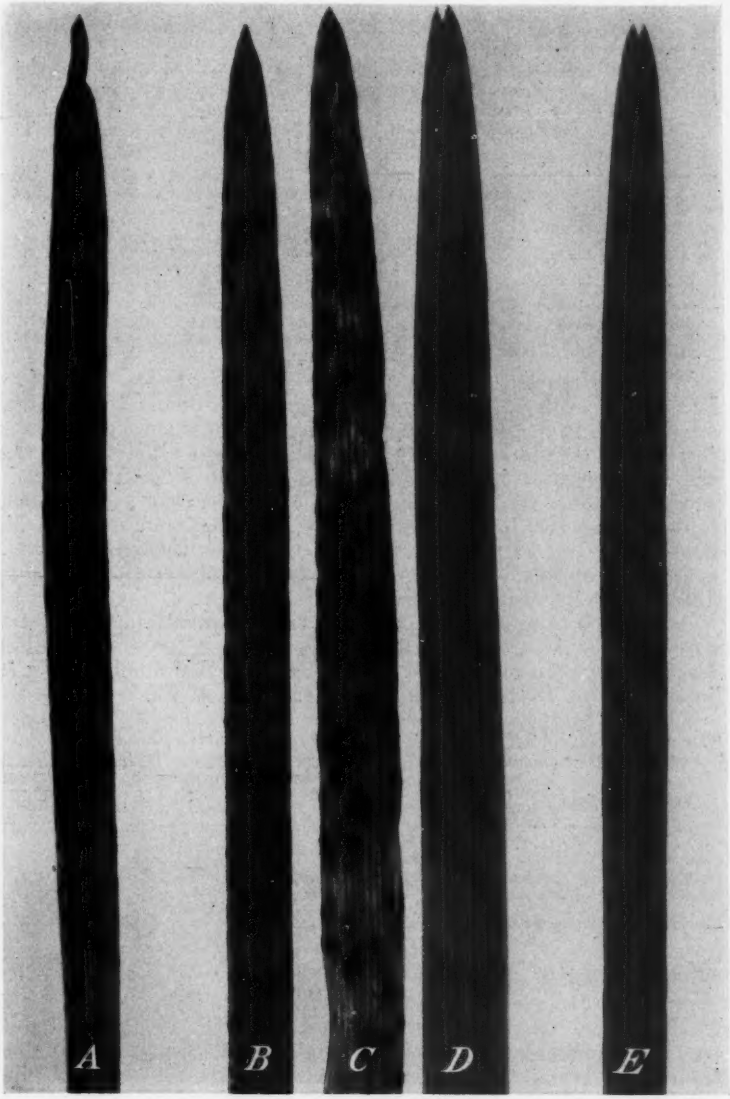

FIGURE 4.—Reaction of primary leaves of seedling plants of the cross Anthony-Bond × Richland-Fulghum and of parental lines to physiologic race 1 of *Puccinia coronata avenae: A*, the susceptible Richland-Fulghum parent; *B* and *C*, susceptible F_3 lines; *D*, a resistant F_3 line; *E*, the resistant Anthony-Bond parent.

TABLE 5.—*Reaction to crown rust of 940 F_2 plants of the cross Anthony-Bond × Richland-Fulghum grown in the field at Manhattan, Kans., and the breeding behavior of seedlings of the F_3 lines tested with Puccinia coronata avenae in the greenhouse*

Reaction of F_2 plants [1]	Reaction of F_3 lines [2]		
	Highly resistant	Segregating	Susceptible
	Number	*Number*	*Number*
Highly resistant (120)	27	93	0
Moderately resistant (190)	6	183	1
Intermediate (158)	0	150	8
Susceptible (472)	0	63	409
	33	489	418

[1] χ^2 for F_2 data based on ratio of resistant to susceptible classes 0.121, range of P 0.50–0.95.
[2] χ^2 for F_3 data based on ratio of highly resistant: segregating: susceptible 3.512, range of P 0.10–0.20.

While segregation for reaction to crown rust in F_2 was determined by readings made only on adult plants grown in the field, the F_3 was studied both in the seedling stage in the greenhouse and in the flowering stage in the disease nursery. The most striking difference between the F_2 segregation in the field and readings on F_3 seedlings in the greenhouse was the complete absence of moderately resistant and intermediate types in the greenhouse. F_3 seedlings were inoculated in the primary-leaf stage of growth and they were either highly resistant or fully susceptible. This simplified greenhouse observations since the differences were so apparent.

In addition to the 940 F_2 plants grown at Manhattan, 1,051 plants were grown at Aberdeen. Thus 1,991 F_3 lines were tested for seedling reaction to crown rust in the greenhouse. Of this number, only 50 lines bred true for resistance, 989 segregated, and 952 bred true for

TABLE 6.—*Breeding behavior of F_3 lines of the cross Anthony-Bond × Richland-Fulghum when inoculated with Puccinia coronata avenae in the greenhouse, compared with the reaction of flowering plants of the same lines to the same rust in the field*

Location	Stage of growth	Observed or expected	F_3 lines				χ^2	Range of P
			Resistant	Segregating	Susceptible	Total		
			Number	*Number*	*Number*	*Number*		
Field	Flowering	O	50	1,002	939	1,991	0.337	0.50–0.95
Greenhouse	Seedling	O	50	989	952	1,991	.474	.50–.95
		E	54	906	941	1,991		

susceptibility (table 6). In segregating lines all types of segregation, from those containing mostly resistant plants to those containing mostly susceptible plants, appeared.

The F_3 field and greenhouse reactions were similar in that the same lines bred true for resistance and susceptibility and the same lines segregated in the greenhouse and in the field (table 6). However, moderately resistant and intermediate types appeared in segregating lines in the field. By making counts in certain segregating lines in the field it was concluded that the moderately resistant types were

highly resistant in the seedling stage while the intermediate types were fully susceptible. The inheritance of resistance to crown rust in this cross is explained on a four-factor basis in which two sets of complementary factors were interacting. A factorial analysis of resistant and segregating lines based on this hypothesis is shown in table 7.

TABLE 7.—*Factorial analysis of homozygous resistant and segregating lines in F_3 of the cross Anthony-Bond × Richland-Fulghum showing seedling and flowering plant reactions to Puccinia coronata avenae expected on the basis of a 4-factor difference* [1]

F_2 genotype	Phenotypic expression [2] in—			
	Seedling plants		Flowering plants	
	F_3 plant	F_3 line	F_3 plant	F_3 line
AABBCCDd	S	1R:3S	I	1R:2I:1S.
AABBCcDD	S	1R:3S	I	1R:2I:1S.
AABBCcDd	R	11R:5S	MR	7R:4MR:4I:1S.
AABbCcDD	S	3R:13S	I	3R:6I:7S.
AaBBCcDD	S	3R:13S	I	3R:6I:7S.
AABbCCDd	S	3R:13S	I	3R:6I:7S.
AaBBCCDd	S	3R:13S	I	3R:6I:7S.
AABbCcDd	R	33R:31S	MR	21R:12MR:12I:19S.
AaBBCcDd	R	33R:31S	MR	21R:12MR:12I:19S.
AaBbCCDd	S	9R:55S	I	5R:4MR:18I:37S.
AaBbCcDD	S	9R:55S	I	5R:4MR:18I:37S.
AaBbCcDd	S	83R:173S	I	35R:48MR:52I:121S.
AABBCCdd	R	R	R	R.
AABBCcdd	R	R	R	R.
AaBBCCdd	R	3R:1S	R	3R:1S.
AABbCCdd	R	3R:1S	R	3R:1S.
AaBBCcdd	R	3R:1S	R	3R:1S.
AABbCcdd	R	3R:1S	R	3R:1S.
AaBbCCdd	R	9R:7S	MR	5R:4MR:7S.
AaBbCcdd	R	9R:7S	MR	5R:4MR:7S.
AABBccDD	R	R	R	R.
AABBccDd	R	R	R	R.
AaBBccDD	R	3R:1S	R	3R:1S.
AABbccDD	R	3R:1S	R	3R:1S.
AaBBccDd	R	3R:1S	R	3R:1S
AABbccDd	R	3R:1S	R	3R:1S.
AaBbccDD	R	9R:7S	MR	5R:4MR:7S.
AaBbccDd	R	9R:7S	MR	5R:4MR:7S.
AABBccdd	R	R	R	R.
AaBBccdd	R	3R:1S	R	3R:1S.
AABbccdd	R	3R:1S	R	3R:1S.
AaBbccdd	R	9R:7S	MR	5R:4M7S.

[1] *A* and *B* are complementary factors for resistance carried by Anthony-Bond. *C* and *D* are complementary inhibitors of the expression of *A* and *B* and are carried by Richland-Fulghum. Only 32 genotypes are shown. The remaining 49 bred true for susceptibility.

[2] The symbols R, MR, I, and S are phenotypic symbols denoting high resistance, moderate resistance, intermediacy, and susceptibility, respectively.

Since the four phenotypic classes appeared only in the reaction of mature plants, the following discussion of gene interaction applies to the mature plant. It is assumed that two dominant complementary factors (*A* and *B*) for resistance are carried by the Anthony-Bond parent. These factors are inherited independently and, because of their complementary nature, both must be present to give a plant the expression of the Bond type of resistance. When both of these factors are heterozygous (*AaBb*), the plant is not highly resistant but is only moderately resistant in the adult stage. However, if both dominant factors are homozygous (*AABB*), or if one is homozygous and the other heterozygous (*AABb* or *AaBB*), the plant shows the Bond type of resistance to crown rust. Plants breeding true for resistance

must be homozygous for both dominant factors (*AABB*). If either or both of these factors are recessive (*aaBB*, *aaBb*, *AAbb*, *Aabb*, *aabb*) the plant is fully susceptible and will breed true for susceptibility.

The inheritance in the cross studied would be relatively simple if only the two complementary factors *A* and *B* were involved. However, the inheritance has been complicated by the action of two other independently inherited dominant complementary factors, *C* and *D* from the Richland-Fulghum parent, which when present together, are capable of inhibiting the expression of the factors for resistance. The degree to which they inhibit the expression of resistance is determined by two conditions: (1) The number of dominant inhibiting genes present, and (2) the number of dominant genes for resistance present. It is possible for two, three, or four dominant inhibiting genes to be present and show an inhibiting effect, depending on whether the plant is heterozygous for both (*CcDd*), heterozygous for one (*CcDD*,*CCDd*) or homozygous for both (*CCDD*). If homozygous for both, four dominant inhibiting genes are acting, masking the expression of any combination of genes for resistance and giving a fully susceptible reaction. The progeny of any plant which carries both of the inhibitors in a dominant homozygous condition will breed true for susceptibility to crown rust regardless of the genes for resistance (*A* and *B*). Thus *AABBCCDD* would breed true for susceptibility.

If a plant is heterozygous for one inhibitor and homozygous for the other (*CCDd* or *CcDD*), it carries three dominant inhabiting genes whose inhibiting effect on any combination of the genes for resistance gives an intermediate-type reaction when both genes for resistance are present.

If a plant is heterozygous for both inhibitors (*CcDd*), the inhibitors show their weakest inhibiting effect because only two domiant genes are present. The inhibitors in this doubly heterozygous condition change the expression of the genes for high resistance (*AABB*, *AaBB*, *AABb*) to that of moderate resistance in the mature plant. The interaction of these four genes all in a heterozygous condition (*AaBbCcDd*) results in an intermediate reaction in mature plants.

Since the moderately resistant and intermediate types appeared among mature plants of the F_2 and F_3 generations in the field and did not appear in the seedling plants of a similar F_3 generation in the greenhouse, it follows that the types appearing as moderately resistant or intermediate in the field appeared either as highly resistant or susceptible in the greenhouse where a sharp segregation occurred. In order to determine how these genotypes were expressed phenotypically in the seedlings, the rust reactions of about 100 selected segregating F_3 lines were observed in the seedling and in the mature-plant stage. From these observations it was concluded that all types appearing as moderately resistant in the mature plant appeared as highly resistant in the seedling. Similarly, all types appearing as intermediate in the field appeared as susceptible in the seedling stage.

Segregation of the F_2 generation in the field on the proposed four-factor hypothesis would theoretically result in a ratio of 35 highly resistant plants : 48 moderately resistant : 52 intermediate : 121 susceptible. When placed on a basis of the 940 plants actually grown: a ratio of 129 resistant: 176 moderately resistant: 191 intermediate: 444 susceptible would be expected. Actually a ratio of 120 resistant:

190 moderately resistant : 158 intermediate : 472 susceptible was observed. When the resistant and moderately resistant plants were placed in one class and the intermediate and susceptible in another class, a ratio of 310 resistant to 630 susceptible plants was obtained as shown in table 5. The observed F_2 ratio, therefore, closely fits the expected ratio as shown by the χ^2 test.

According to the foregoing hypothesis, only 54 of the F_3 lines would be expected to breed true for resistance. Actually, 50 lines were observed in both the greenhouse and the field to be breeding true for resistance (table 6). Theoretically, exactly half of the population (996 lines) would be expected to segregate. In the greenhouse 989 lines appeared to segregate, while 1,002 seemed to be segregating in the field. According to the hypothesis, 941 lines were expected to breed true for susceptibility. Actually, 952 lines in the greenhouse and 939 lines in the field appeared to be breeding true for susceptibility.

The close agreement between observed and expected ratios in both F_2 and F_3 (table 5) is further substantiated by the plant counts made in segregating F_3 lines in the greenhouse. The number of resistant and susceptible plants expected in the various segregating groups can be added to give numbers which represent the segregation as a whole. If the F_3 segregating lines are considered collectively, 46.24 percent of the seedlings would be expected to be resistant and 53.76 percent susceptible. There were 12,973 plants counted in 440 consecutive segregating F_3 lines. Of this number 6,137 (47.3 percent) were resistant, and 6,836 (52.7 percent) were susceptible. The agreement between the observed and expected percentages, therefore, appears to be good considering the many different types of segregation that occurred and the great variation in the number of plants in each of the 440 segregating F_3 lines.

This four-factor hypothesis confirms the reports of Hayes, Moore, and Stakeman,[15] Hayes,[16] and Weetman,[17] who state that the crown rust reaction of Bond is controlled by two complementary factors. It also shows how a fairly simple inheritance may be complicated by the action of an additional set of complementary factors. The hypothesis is well substantiated by the close agreement between observed and expected numbers in the F_2 and F_3 generations. The analysis of 440 segregating F_3 families adds even further proof, since all expected types of segregation were observed. Certain selected lines studied in the F_4 generation segregated or bred true for susceptibility or resistance as expected.

RELATION OF CROWN RUST, STEM RUST, AND SMUT REACTION

From both scientific and practical points of view it semed desirable to ascertain whether there was any association between the factors for resistance to any of the three diseases. No relationship was noted between stem and crown rust inheritance in the Fulghum-Victoria × Richland-Fulghum and Fultex × Richland-Fulghum crosses as the observed 9:3:3:1 F_2 ratio was close to the expected χ^2 being 2.246 and P within the range of 0.50–0.95. In the Anthony-Bond × Richland-Fulghum cross, the x^2 on F_3 data for the expected ratio showing

[15] See footnote 4, p. 45.
[16] See footnote 5, p. 45.
[17] See footnote 11, p. 52.

no linkage was 10.817 (8 degrees of freedom) with the range of P being 0.20–0.30.

Smut resistance appeared to be distributed at random among the various classes of rust reaction.

Victoria and Bond types of reaction to crown rust show differences in their degree of resistance. The resistance of Bond approaches immunity, showing only a slight flecking at points of infection, while the less desirable resistance of Victoria is expressed by small pustules surrounded by necrotic areas. Weetman [18] has reported that the resistance of Bond to race 1 is conditioned by two dominant complementary genes and that the resistance of Victoria to the same race is due to one factor distinct from the Bond genes.

This investigation has confirmed Weetman's report in regard to the nature and number of factors involved in the inheritance of each type of resistance. Since both types of resistance were not involved in any one of the four crosses studied in this investigation it was impossible to determine whether the two types of resistance were inherited independently. However, some knowledge of this was gained by observing the F_2 segregation of certain crosses, Fultex × Bond-Fulghum and Fultex × D 69-Bond, that were grown in the disease nursery in 1942. Three main types of crown rust reaction were observed. Approximately 37 percent of the plants had the Victoria type of resistance, 49 percent had the Bond type of resistance, and 14 percent were susceptible. The respective percentages expected would be 33, 56, and 11, if it is assumed that the three factors involved were independently inherited and that the Bond type masked the Victoria type of resistance when all three dominant factors were present in the F_2 plants. Therefore the data obtained seem to indicate that the factor determining Victoria resistance is distinct from the two governing the resistance of Bond.

SUMMARY

The inheritance of reaction to a mixture of races of *Ustilago avenae* and *U. levis*, *Puccinia graminis avenae* physiologic races 2 and 8 and *P. coronata avenae* physiologic race 1 was studied in four crosses, Richland-Fulghum × Fulghum-Victoria and reciprocal, Fultex × Richland-Fulghum, and Anthony-Bond × Richland-Fulghum in the field and in the greenhouse. The disease reaction of the first three crosses was the same, but that of the fourth cross was different because of the genetic constitution of the Anthony-Bond parent.

Transgressive segregation for smut susceptibility occurred in the first three crosses. The parents of these crosses were all highly resistant to the smut inoculum, showing no infection. The reaction of the hybrids in the F_2 and F_3 generations indicated that each parent carried a dominant factor for resistance. When these two independently inherited duplicate dominant factors were both absent in a hybrid, the plant was susceptible to smut.

In the Anthony-Bond × Richland-Fulghum cross a similar condition existed except that there was one dominant factor for high resistance to smut and one for moderate resistance.

[18] See footnote 11, p. 52.

Resistance to stem rust was dominant in all four crosses and appeared to be governed by a single factor.

Reaction of the three crosses involving Fulghum-Victoria and Fultex to crown rust was governed by one factor with resistance dominant.

The crown rust reaction of Authony-Bond × Richland-Fulghum was controlled by the interaction of four factor pairs, two sets of dominant complementary factors. One set, dominant complementary genes for resistance, was carried by Anthony-Bond, the resistant parent, and the other set, dominant complementary inhibitor genes epistatic to the genes for resistance was carried by Richland-Fulghum, the susceptible parent. The expected F_2 ratio was 35 fully resistant : 48 moderately resistant : 52 intermediate : 121 fully susceptible plants. In the seedling stage in the greenhouse the moderately resistant plants were fully resistant, while the intermediate plants were fully susceptible. The expected F_3 segregation was 7 lines breeding true for resistance, 128 lines segregating, and 121 lines breeding true for susceptibility. Observed ratios in F_2 and F_3 proved to be statistically near the expected ratios.

No association between the factors determining the reaction to different diseases was observed in any of the crosses studied.

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